

Standardized Interim Progress Report

A. Project Identifiers

- 1) Award Number: NA16FX1418
- 2) Grant Program/CFDA#: 11-439
- 3) Name of Recipient Organization: Mystic Aquarium
- 4) Principal Investigator: Lisa M. Mazzaro
- 5) Project Title: Investigation of Retinol (vitamin A) and Tocopherol (vitamin E)
Status in Steller Sea Lions: Contribution to Nutritional Stress in Declining Populations
- 6) Funding: Federal \$421,690 Match \$84,000
- 7) Award Period: From: July 1, 2001 To: June 30, 2003
- 8) Period Covered by this Report: From: July 1, 2001 To: December 31, 2001

B. Project Summary

Nutritional stress has been identified as having a significant, if not primary, role in the precipitous decline of the Steller sea lion in parts of its range (Marine Mammal Commission Annual Reports). Investigations into this hypothesis have focused on food availability (Merrick *et al.* 1997), caloric composition (Rosen and Trites 2000a), and various energetic concerns arising from both these issues (Rosen and Trites 1999, 2000b). The lower fat content and higher digestive costs of a pollock diet resulted in weight loss in captive animals, lending credence to the argument that dietary shifts necessitated by depleted stocks of energy-rich prey such as herring may be having population-wide effects on Steller sea lions. Though food quality is recognized as an important issue in the consideration of nutritional stress (Rosen and Trites 2000a,c), little attention has been directed to specific nutrients that might bear on health and productivity. Chief among these are the fat-soluble vitamins, which assume particular importance in view of the recognized differences in the fat content between the preferred and present diets of Steller sea lions.

Research into the specific vitamin requirements for marine mammals is sparse (Geraci 1981). In captivity, supplements are generally provided to compensate for a reduced variety of foodstuffs and losses during frozen storage of the diet. Often, the level of supplementation is established empirically, sometimes with negative consequences (Mazzaro *et al.* 1995a). Of necessity, studies on the metabolic consequences of various diets on Steller sea lions have been conducted on animals receiving multi-vitamin supplements (Rosen and Trites 1999). There is no published information on the vitamin content of the natural diet, the vitamin status of free-ranging Steller sea lions, or the metabolic parameters that define their vitamin requirements. We are investigating these aspects of Steller sea lion health with respect to the fat-soluble vitamins A (retinol) and E (tocopherol).

Mystic Aquarium has exhibited Steller sea lions continuously since 1976, and is one of only three facilities in the United States currently maintaining these animals. Long experience with the husbandry of this species, and recent research projects undertaken there (Hirons *et al.* in review), places the institution at the forefront for conducting the type of investigation described in this proposal. Mystic Aquarium also represents a significant opportunity to inform over 800,000 visitors annually of the NMFS-sponsored

research efforts undertaken in an effort to foster the recovery of Steller sea lion populations.

STUDY OBJECTIVES

1. Establish the status of free-ranging Steller sea lions with respect to the fat-soluble vitamins A (retinol) and E (tocopherol) by analyzing blood and tissue samples.
2. Determine the vitamin A and E content of natural food items for Steller sea lions.
3. Determine the metabolic requirements of Steller sea lions for vitamins A and E by relating intake to blood levels in captive specimens.
4. Measure metabolic clearance rates of vitamins A and E using isotope tracers and vitamin analogs, to further refine estimates of daily requirements in captive Steller sea lions.

Examine changes in vitamin A and E status in relation to various life history stages (e.g. pregnancy, lactation, early development) in captive Steller sea lions.

C. Summary of Progress and Results

1) Scheduled Tasks

a) Objective 1

- Make contacts at ADF&G and NMML to acquire free-ranging Steller Sea Lion samples during 2001 collection trips
- Make arrangements for shipping samples to Mystic Aquarium
- Analyze samples

b) Objective 2

- Make contacts for acquiring natural prey samples
- Make arrangements for shipping samples to Mystic Aquarium
- Develop working method for fish analysis
- Analyze samples

c) Objective 3

- Make arrangements with ASLC, Vancouver Aquarium, Oregon Coast Aquarium and Dolfinarium Harderwijk for collection of captive SSL serum samples
- Collect serum samples from captive SSL at Mystic Aquarium
- Analyze diets of captive SSL for vitamin A and E levels

d) Objective 4

- Plan isotope tracer studies at Mystic Aquarium
- Initiate talks with ASLC and Vancouver Aquarium for use of their animals for tracer studies
- Complete first tracer study at Mystic Aquarium
- Analyze samples from first tracer study and use to adjust sampling times and doses for future studies

2) Activities

a) Objective 1

- The following samples from SSL pups < 2 months of age were collected and analyzed for serum retinol, tocopherol, cholesterol and triglyceride

There have been no changes to the goals/objectives of this study

4) Results and/or specific products prepared during the reporting period.

a) Objective 1

Blood samples were collected by researchers with the Alaska Department of Fish and Game (ADF&G) and the National Marine Mammal Lab (NMML) from Steller sea lion pups on rookeries in the Central and Eastern Aleutian Islands, Southeast Alaska and Gulf of Alaska. All pups were less than 2 months old. Serum samples (n=110) were analyzed for retinol, alpha-tocopherol, cholesterol and triglycerides. High performance liquid chromatography was used for vitamin analysis and commercially available test kits from Sigma Chemical were used to perform lipid analysis. Vitamin E in the blood is carried by lipoproteins and is affected by the intake of vitamin E and by blood lipid levels. For these reasons vitamin E is usually reported as E/lipid ratio. Statistix software was employed in all statistical analyses. Within the eastern stock, samples were collected from 2 different islands. Analysis of variance showed no significant differences in any of the parameters between the locations. Samples from the western stock were examined by area (central and eastern Aleutians, Gulf of Alaska) and also by island. Again there were no significant differences in any of the measured parameters. When data were analyzed by stock (eastern vs. western), there were a significant differences in tocopherol and triglyceride levels, with the western stock showing higher concentrations. Serum retinol (0.46 ± 0.11 $\mu\text{g/ml}$) and alpha-tocopherol (19.82 ± 4.03 $\mu\text{g/ml}$) levels were within presumed 'normal' ranges compared to both captive and free-ranging levels for other pinniped species. There were also no correlations between vitamin E and either cholesterol or triglyceride levels suggesting that there is a true difference between vitamin E levels in the two populations that can not be explained by lipid levels. Correlations between vitamins and weight or length data were also not present.

Currently an abstract is being prepared and will be submitted for presentation at the annual meeting of the International Association for Aquatic Animal Medicine.

b) Objective 2

None at this time

c) Objective 3

None at this time

d) Objective 4

Data from vitamin tracer study have been analyzed and provided information that will be used to refine the study for subsequent animals. First, we found that the dose of both the labeled vitamin E and the vitamin A2 were

Number of Animals	Stock	Island
19	East	Lowrie
11	East	Hazy
9	East	Grassy
10	West	Seguam/Saddle Ridge
4	West	Akutan/Cape Morgan
13	West	Ugamak/North
13	West	Ugamak/South
7	West	Chowiet
7	West	Fish (Wooded)
17	West	Seal Rocks/PWS
Total = 110		

- Samples from juvenile SSL have been collected during fall and winter trips and will be sent in early 2002.

b) Objective 2

- Working method for fish sample analysis has been developed
- Samples of ground prey representing diet of the eastern stock of SSL from season 1 have been received from Auke Bay Lab and will be analyzed in early 2002. Future samples are in the process of being ground and will be shipped when ready.
- Samples of ground prey representing diet of the western stock of SSL are being prepared at UAF, and will be shipped to Mystic Aquarium when ready.

c) Objective 3

- Arrangements have been made with ASLC and Vancouver Aquarium for collection of serum samples from collection animals
- Dolfinarium Harderwijk is in the process of training animals for blood collection
- Oregon Coast Aquarium is not currently bleeding their animals
- Sampling from collection animals at Mystic Aquarium will begin in 2002
- Diet analysis of Mystic animals will begin in 2002

d) Objective 4

- The first SSL tracer study at Mystic Aquarium was completed in November. Samples were analyzed and data is being looked at to determine if any changes need to be made before subsequent animals undergo the study.
- At the Biennial Conference on the Biology of Marine Mammals, held in Vancouver in November, 2001, we met with staff from ASLC and Vancouver Aquarium to discuss plans for utilizing their animals for future tracer studies. Both organizations expressed interest, but specific dates for the trials were not set. Continued discussions are underway.

3) Changes to the goals/objectives during the reporting period

adequate. Second, we found that the sampling times provided information about absorption rate and peak levels in blood, but recognized that altered sampling times will improve the data from subsequent trials. Third, we determined the need to continue sampling for a longer time period because the blood levels did not return to baseline levels after 1 week. We are currently working on data manipulation and curve fitting.

D. Problems

- 1) Circumstances of problems that prevented completion of a scheduled task.
Describe consequences resulting from inability to complete a given task.

Objective 2 (analysis of prey samples) is slightly behind schedule because method development took longer than expected and equipment failures caused additional problems

- 2) Actions/activities taken to resolve the problem

The method development has been worked out and equipment problems resolved. We should be able to begin running these samples this month and will be caught up soon.

- 3) Special problems or differences between budgeted and actual expenditures

None at this time